

# Hereditary Protein S Deficiency in Young Adults with Arterial Occlusive Disease

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## Summary

Protein S is the vitamin K dependent cofactor of activated protein C. It has an important role in the regulation of blood coagulation and fibrinolysis. Hereditary protein S deficiency is associated with familial venous thrombophilia. Since a few patients with arterial occlusions have been reported to be protein S deficient, it is speculated that hereditary protein S deficiency may be also a risk factor for the development of arterial thrombosis. In a group of 37 consecutive patients with arterial occlusive disease presenting before the age of 45, three patients were found heterozygous for hereditary protein S deficiency. None of the patients had a protein C deficiency or an antithrombin III deficiency. Family investigations showed a clear association between the hereditary deficiency and venous thrombosis, but a relation between the deficiency and arterial thrombosis was less obvious. A review of previous literature on patients with arterial thrombosis and protein S deficiency revealed that more extensive studies are needed to demonstrate whether or not hereditary protein S deficiency is a risk factor for the development of arterial thrombosis.

## Introduction

Protein S is a vitamin K dependent plasma protein that was discovered in 1977 (1). Since 1980, evidence for its role as an essential cofactor of activated protein C has been reported in several papers, where its importance in the expression of both the anticoagulant and the profibrinolytic activity of activated protein C was demonstrated (2–4). After the first case reports of patients with familial venous thrombophilia due to hereditary protein C deficiency (5–7), it was pointed out in subsequent papers that this syndrome could also be caused by hereditary protein S deficiency (8–10).

Moreover, some investigators have reported the presence of hereditary protein S deficiency in young patients with manifestations of arterial thrombosis (11–18).

To compare the prevalence of protein S deficiency in patients with arterial thrombosis with the reported prevalence in patients with venous thrombophilia, we screened 37 consecutive patients with arterial occlusion presenting before the age of 45. In the families of the patients with a hereditary deficiency we investigated whether the defect was associated with symptoms of arterial thrombosis, venous thrombosis or both. In this paper we present the results of these studies and discuss possible relations between protein S deficiency and the occurrence of venous and arterial thrombosis.

## Patients and Methods

We carried out a study in the department of vascular surgery in the University Hospital Leiden, with the purpose to detect disorders in the regulation of coagulation and fibrinolysis, fat metabolism and methionin metabolism in patients with arterial occlusions in the lower extremities presenting before the age of 45. Patients with vascular occlusions caused by trauma, popliteal entrapment or adventitial cystic disease were excluded from the study. Thirty-seven consecutive patients who had been treated at the department of vascular surgery between the years 1978 and 1987 were invited to the outpatient clinic and all agreed to participate in the study. The diagnosis was based on the patient's history, the physical examination, the Doppler pressure indices at the ankle level, and if available the angiographic findings. When surgery was performed, the intra-operative observations and the histologic examination of the specimen obtained during surgery confirmed the diagnosis. The lesions were equally distributed between aortic-iliac and femoro-distal regions, as could be seen on the angiograms.

All laboratory methods and outcomes of the complete study have been published in detail (19). To detect an isolated protein S deficiency venous blood was collected in 1/10 volume of 0.11 M trisodium citrate. Platelet free plasma was obtained by centrifuging the platelet poor plasma for 30 min at 20,000 × g at 4° C. Total protein S antigen was measured with an ELISA, recently developed in our laboratory (20) and based on the same principles earlier applied in the immunoradiometric assay (21). Protein C antigen, factor II antigen, and factor X antigen were measured by electro-immunoassay (6). From the three patients where a low plasma level protein S was found (see below) a second plasma sample was obtained to confirm the first results. The results of the second analysis confirmed that all three patients had an isolated protein S deficiency. The diagnosis was based on the following criteria: a total protein S-antigen level below the lower limit of the normal range found in healthy controls (67–125%, n = 45) or, for patients on oral anticoagulant therapy, a total protein S-antigen level below the lower limit of the range found in a reference group of patients receiving coumarin therapy (33–74%, n = 93). Other vitamin K-dependent factors had to be within the normal range as found in the controls with or without coumarin therapy (21).

Subsequently, all available relatives of the probands were investigated, using the same methods and criteria. In family A and C, with a positive family history for arterial thrombosis, cholesterol levels in serum were measured as well.

The medical history of all participating family members was taken and information about their deceased relatives was also obtained. We classified all family members into three groups according to the probability that they were heterozygous for hereditary protein S deficiency. This classification was based on the laboratory results of the participating family members and the position in the pedigree of those who couldn't participate. Individuals with a documented protein S deficiency have a 100% probability to be heterozygous, those with normal protein S levels have a 0% probability. When their protein S levels are unknown, each parent of a known heterozygote has a 50% probability to be heterozygous for the deficiency as well, aunts and uncles have a 25% probability and so on. Group 1 includes family members with a less than 12.5% probability to be heterozygous, group 2 represents all of those with a probability between 12.5% and 50%, and individuals with a 100% probability to be heterozygous are in group 3. We drew up life tables for the occurrence of venous thrombosis and arterial thrombosis.

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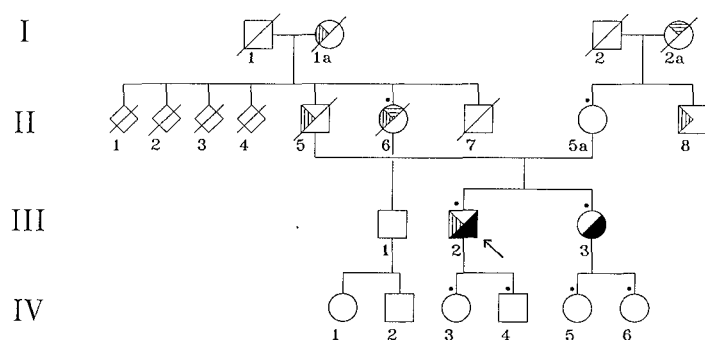


Fig. 1 Pedigree of family A. □ = male; ○ = female; ◇ = sex unknown; ▤ = history of arterial thrombosis; ▥ = history of venous thromboembolism; ▦ = deceased; ▧ = investigated; ▨ = heterozygous for hereditary protein S deficiency; ▩ = proband

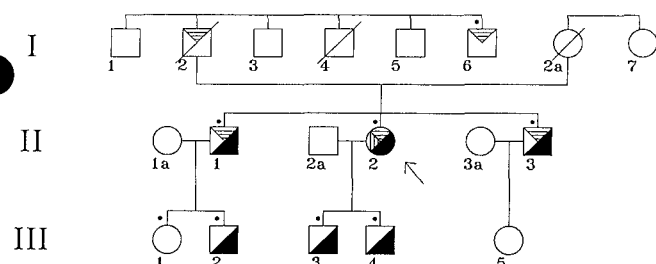


Fig. 2 Pedigree of family B. Symbols: see Fig. 1

## Results

### Case Reports and Family Results

In the group of 37 consecutive patients with peripheral arterial occlusions presenting before the age of 45, three patients (8%) were protein S deficient. Family investigations were then carried out. The results of the measurement of protein S, protein C, factor II and factor X antigen have been listed in Tables 1, 2 and 3. The medical histories of the three patients and the results of the family studies are the following:

Patient A (III-2 in Fig. 1) had the first complaints of claudication at the age of 39, which were slowly progressive in the following year. He smoked about 10 cigarettes a day, was healthy, and hypertension and diabetes mellitus were absent. Angiography showed an occlusion of the right superficial femoral artery and a venous femoropopliteal bypass was performed. Five years later he was free of symptoms with normal Doppler ankle/arm indices. When he entered the study, a serum cholesterol level was measured of 8.9 mmol/l. Further investigations showed that he had a type IIb hyperlipoproteinemia. In his family (family A, Fig. 1) II-5 and I-1a had died of a myocardial infarction before the age of 50. II-6 had suffered from recurrent superficial thrombophlebitis, with the first episode after delivery at the age of 35. She survived two myocardial infarctions at 68 and 69 years of age, but died recently, presumably of a third myocardial infarction. I-2a was said to have had deep venous thrombosis after childbirth. II-8 who has non-insulin dependent diabetes mellitus, has recently suffered from an arterial occlusion in a leg. He could not participate in the study. No other family members had suffered episodes of venous thromboembolism or arterial occlusion. Only the asymptomatic sister of the proband (III-3) was found to be heterozygous for protein S deficiency. In this family serum cholesterol levels were measured. The serum cholesterol level was

high (9.6 mmol/l) in II-6, but normal (i. e. less than 6.5 mmol/l) in the other participants.

Patient B (II-2 in Fig. 2) had her first symptoms of claudication at the age of 39. She smoked 15 cigarettes a day and was treated for hypertension. Her medical history further included pulmonary embolisms of unknown origin at the age of 35 and seropositive rheumatoid arthritis since age 26. She had had several surgical operations without complications. Angiography showed an occlusion of the right, and a significant stenosis of the left common iliac artery. An aortobifemoral bypass was implanted and she stopped

Table 1 Family A: results of laboratory studies

Pedigree number	Sex	Age (years)	PS-ag (%)	PC-ag (%)	FII-ag (%)	FX-ag (%)	OAC (+/-)	def (+/-)
II-5a	F	70	116	118	123	118	-	-
II-6	F	76	97	154	123	154	-	-
III-2*	M	44	30	54	54	43	+	+
III-3	F	35	65	117	138	152	-	+
IV-3	F	20	87	123	111	140	-	-
IV-4	M	18	106	79	118	106	-	-
IV-5	F	12	114	87	102	102	-	-
IV-6	F	8	87	87	108	102	-	-

F = female, M = male; OAC = oral anticoagulation; def = protein S deficient; \* proband.

Table 2 Family B: results of laboratory studies

Pedigree number	Sex	Age (years)	PS-ag (%)	PC-ag (%)	FII-ag (%)	FX-ag (%)	OAC (+/-)	def (+/-)
I-6	M	64	102	200	138	118	-	-
II-1	M	45	11	33	32	37	+	+
II-2*	F	43	66	100	145	120	-	+
II-3	M	39	27	74	63	45	+	+
III-1	F	19	86	82	74	60	-	-
III-2	M	17	45	87	120	80	-	+
III-3	M	20	55	115	92	80	-	+
III-4	M	17	49	110	92	95	-	+

F = female, M = Male; OAC = oral anticoagulation; def = protein S deficient; \* the proband.

Table 3 Family C: results of laboratory studies

Pedigree number	Sex	Age (years)	PS-ag (%)	PC-ag (%)	FII-ag (%)	FX-ag (%)	OAC (+/-)	def (+/-)
II-6	M	62	134	94	128	118	-	-
II-9	M	72	79	48	67	61	+	-
III-1	M	61	126	94	87	94	-	-
III-2	M	47	127	108	138	123	-	-
III-3	F	45	103	120	103	130	-	-
III-5	F	51	117	125	132	123	-	-
III-6	M	41	111	125	96	118	-	-
III-8	M	41	101	102	96	103	-	-
III-9	M	58	118	120	102	114	-	-
III-12	M	51	127	108	120	168	-	-
III-13	F	36	111	108	128	123	-	-
III-14	F	34	95	108	96	130	-	-
III-15	M	29	96	96	103	123	-	-
III-16*	M	45	19	68	47	29	+	+
III-17	F	32	93	100	110	118	-	-
III-18	M	21	96	84	90	104	-	-
IV-26	M	11	35	75	71	77	-	+
IV-27	F	7	36	75	81	81	-	+

F = female, M = male; OAC = oral anticoagulation; def = protein S deficient; \* proband.



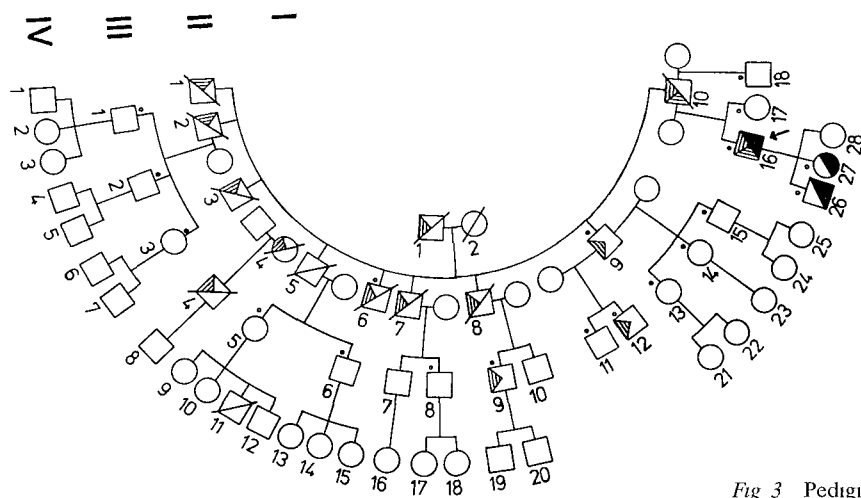


Fig 3 Pedigree of family C Symbols see Fig 1

smoking Four years later she was free of symptoms and the Doppler ankle/arm indices were normal When she entered the study, a serum cholesterol level of 6.2 mmol/l was measured

In her family (family B, Fig 2), I-2 was reported to have suffered from a deep venous thrombosis at the age of 19 after immobilization during a pneumonia Only one of his brothers (I-6) was able to participate in the study At the age of 62, this man had received anticoagulant therapy for an axillary vein thrombosis following abdominal surgery II-1 and II-3 had suffered from spontaneous deep venous thrombosis at the age of 30 None of the family members had had symptoms of arterial occlusive disease II-1 and II-3 were found to be heterozygous for protein S deficiency, as were III-3, III-4 and III-2

Patient C (III-16 in Fig 3) had claudication when he was 35 years of age He smoked 25 cigarettes a day and had always been healthy before A few months earlier he had suffered from a deep venous thrombosis in the left leg after immobilization for what was thought to be a tendinitis, and he received coumarin therapy since Angiography showed an occlusion of the aorta with an extensive collateral circulation An aortobifemoral bypass was performed and 1 year later he was free of symptoms At the start of the study a serum cholesterol level of 8.1 mmol/l was measured In his family (family C, Fig 3) II-10 had suffered from pulmonary embolisms in the year before he died of lung cancer He also had had a myocardial infarction before the age of 65 Of his generation, only two relatives were still alive at the time of the investigation The first was II-6, who had a myocardial infarction at the age of 60 One month after the study he died after a second infarction, aged 62 The second was II-9, who at 56 refused to receive surgery for arterial occlusive disease in a leg and who had a myocardial infarction at the age of 70, while on oral anticoagulant therapy II-1 died of myocardial infarction at age 66 II-2 suffered from a stroke at the age of 42 and died following vascular surgery for an acute arterial occlusion in a leg at the age of 54 II-4 suffered a fatal stroke at age 50 Her son (III-4) died of a heart attack during a soccer match, 33 years old II-7 had had non-insulin dependent diabetes mellitus, he died in his sleep at the age of 66, after having chestpain the previous evening Some years earlier a medical examination had revealed that he had suffered a silent myocardial infarction II-8 had a heart attack at the age of 65 His eldest son (III-9) suffered from an occlusion of the left anterior cerebral artery at age 57 III-12 received surgery because of an arterial occlusion in the leg when he was 20 years old He was free of symptoms since Of all family members participating, only two children of the proband (IV-26 and IV-27) were found to be heterozygous for protein S deficiency The serum cholesterol

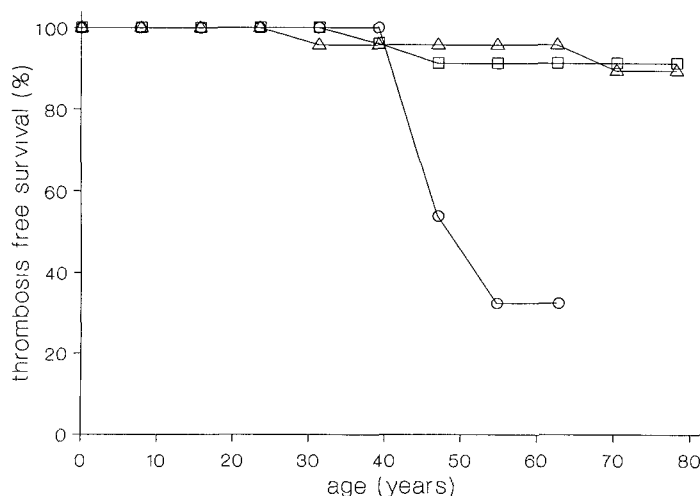


Fig 4 Lifetable venous thromboembolism thrombosis free survival related to the probability of being heterozygous for hereditary protein S deficiency, —□— 0 to 12.5% probability to be heterozygous (n = 52), —△— 12.5 to 50% probability (n = 25), —○— 100% probability (n = 12, probands included)

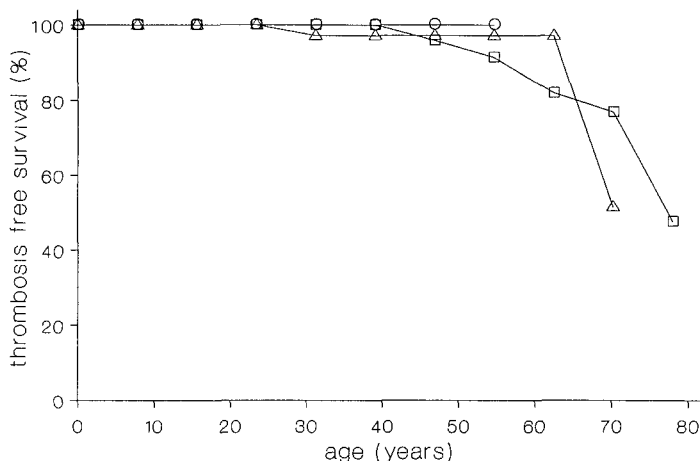


Fig 5 Lifetable arterial thrombosis thrombosis free survival related to the probability of being heterozygous for hereditary protein S deficiency, —□— 0 to 12.5% probability to be heterozygous (n = 52) —△— 12.5 to 50% probability (n = 25) —○— 100% probability (n = 9, probands excluded)



levels in the family were under 6.5 mmol/l, except in II-9 (6.7 mmol/l), III-5 (6.6 mmol/l), III-13 (7.8 mmol/l) and IV-9 (7.1 mmol/l).

After dividing all family members into three groups according to the probability that they were heterozygous for hereditary protein S deficiency we drew up life tables with regard to the occurrence of venous and arterial thrombotic events. After the age of 40, venous thrombotic events occurred more often in heterozygotes than in persons with a 50% or less probability of being heterozygous for the deficiency (Fig. 4). The occurrence of arterial thrombosis did not increase with the probability of having the deficiency (Fig. 5).

## Discussion

Since 1980 the role of protein S as cofactor of activated protein C has been reported in several papers (2–4). Activated protein C inhibits the blood coagulation cascade by inactivating factors Va and VIIIa (22, 23). It accelerates clot lysis as well (4, 24). Protein S is an essential cofactor for the expression of both properties of protein C (3, 4). In plasma protein S is present in a free form and bound in a complex with C4b-binding protein; only the free form acts as a cofactor for activated protein C (25).

In several publications the association between familial thrombophilia and hereditary protein C deficiency was reported (5–7, 26, 27). The same association for hereditary protein S deficiency and venous thrombophilia was demonstrated (8–10). It was established that, in families with venous thrombophilia and protein S deficiency, heterozygotes for the deficiency tend to develop venous thrombosis more often and at a younger age than non-deficient family members (10).

In the literature several patients with arterial thrombosis and protein S deficiency have been reported (8–18). The type of study and the criteria on which the diagnosis protein S deficiency was based showed considerable variation.

Schwarz et al. (9), Collier et al. (11), Mannucci et al. (12), Israels et al. (13) and Girolami et al. (14) all presented a case of a patient with arterial thrombosis at a young age with a hereditary protein S deficiency. No information was given on the selection procedure of the probands. The diagnosis was based on the finding of low total protein S levels (9, 12, 14) or on the presence of low free protein S levels with normal total protein S levels (11, 13). Family investigations revealed heterozygous relatives, some of whom had suffered from venous thromboembolic events (9, 11, 12, 14). No information was given about a possible family history of arterial thrombosis.

Von Felten et al. (15), Thommen et al. (16), Schäfer et al. (17), and Chancellor (28) screened groups of patients with arterial thrombosis for protein S deficiency and other deficiencies. The inclusion criteria of these studies were not always clearly defined. The patients initially reported by von Felten et al. were included in the 33 patients with arterial cerebral thrombosis studied by Schäfer et al. In total nine patients were reported to have a protein S deficiency, defined as low total protein S levels with low free protein S levels, which was familial in four cases. Four other patients had low free protein S levels but normal total protein S levels, and this type of deficiency was found to be familial in one case. Thommen et al. (16) investigated seven patients with arterial thrombosis, and reported one patient with low free protein S levels and low (calculated) total protein S levels, a phenotype also found in an asymptomatic sister of the patient. Other patients were described with low free protein S levels and various outcomes in the calculation of total protein S and the measurement of C4b-BP-bound protein S, but in none of these cases a hereditary defect was proven. In the three reports the

information about the occurrence of venous and arterial thrombosis in the families is incomplete. Chancellor et al. (28) measured total and free protein S antigen levels and found no protein S deficiencies in a study in 38 consecutive patients with unexplained non-hemorrhagic cerebral infarctions.

Recently, Sié et al. (18) reported on six patients with protein S deficiency and arterial thrombosis at a young age, identified among 23 symptomatic heterozygotes from 17 families. The diagnosis was based on a low total protein S level, or on a normal total protein S with a low free protein S antigen level. It is not clear on which criteria the families had been selected and the total number of heterozygotes in these families is unknown. In three of the six patients a hereditary deficiency was not proven or not reported. A positive family history for venous thrombosis was present in three cases, familial arterial thrombosis was not reported.

Only one report on hereditary protein S deficiency in patients selected on the basis of venous thrombophilia gives information about the occurrence of arterial thrombosis in the families studied: Engesser et al. (10) interviewed 72 heterozygotes in 12 protein S deficient families. Twenty-five of these persons were more than 50 years old at the time of the interview (personal communication) and none of them had had symptoms of arterial disease.

We carried out a study in the department of vascular surgery in 37 consecutive patients with symptomatic arterial occlusive disease before the age of 45. Three proved to be heterozygous for hereditary protein S deficiency (8%). The prevalence of hereditary protein S deficiency in the general population is unknown, in patients with venous thrombophilia it is reported to be between 2 and 8% (29–31). The fact that we find a similar prevalence in the group of patients with arterial thrombosis suggests that the defect may be a risk factor for arterial thrombosis as it is for venous thrombosis. However, since the defect is hereditary, an association between deficiency and symptoms should then also be evident in family studies.

To investigate whether or not a relation between protein S deficiency and symptoms of arterial thrombosis was present in the families of the three patients with the deficiency, we divided all family members into three groups according to the probability that they were heterozygous. Life table analysis demonstrated a clear relation between the occurrence of venous thrombosis and a heterozygous state for hereditary protein S deficiency (Fig. 4). This corroborates previous findings in a study of 12 families with hereditary protein S deficiency (10). The result is all the more of interest since, in contrast to the previous study, venous thrombophilia was not a selection criterion in our study.

On the other hand, the occurrence of arterial thrombosis did not increase with the probability of having the deficiency (Fig. 5). However, in this exercise we did not include the three probands since their symptoms were the reason why they were included in the study. Group 3 thus consists of only nine individuals with a 100% probability to be heterozygous. The fact that they were on average younger (mean age 26.7 years, range 8 to 52 years) than the members in the other two groups (mean age 37.1 years, range 0 to 78 years) might explain why no correlation between protein S deficiency and arterial thrombosis was found.

None of the patients in our study had a deficiency of protein C or antithrombin III. This suggests that there might be a special relation between protein S deficiency and the development of arterial thrombi. A direct effect may be possible, as both endothelial cells and platelets are sites of protein S synthesis and action *in vivo* (32). A high serum cholesterol may be a potentiating factor.

We conclude that hereditary protein S deficiency is relatively frequent among patients with arterial occlusive disease. The





defect is a risk factor for the development of venous thrombosis, and it seems to be associated with the occurrence of arterial thrombosis as well. An open eye for this possibility in the approach of individual patients, and more extensive family and epidemiologic studies may help to determine whether or not hereditary protein S deficiency is indeed a risk factor for the development of arterial occlusive disease and thrombosis.

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